CORRESPONDENCE

PRESERVATION OF CORNEAL GRAFTS BY FREEZING

To the Editorial Committee of the BRITISH JOURNAL OF OPHTHALMOLOGY

SIRS,—Mr. Rycroft, in his interesting article "Three Unusual Corneal Grafts" (Rycroft, 1957), describes a successful case of perforating keratoplasty using donor material which had been stored for one month in the deep-freeze at -79°C . He states that this establishes *prima facie* evidence that long-term storage at low temperatures is possible, that modification of the deep-freeze technique has given encouraging results, and that in his opinion the deep-freeze method will become the bank method of the future.

Whilst agreeing on the future possibilities of the deep-freeze method for the preservation of corneal tissue, I believe that the present state of our knowledge regarding the use of such material should be clearly defined. Eastcott, Cross, Leigh, and North (1954), following the work of Polge, Smith, and Parkes (1949), investigated the preservation of corneal tissue by freezing and recorded a series of five lamellar and seven full-thickness grafts using tissue which had been stored at -79°C . after immersion in 15 per cent. glycerol saline. All the lamellar grafts were entirely successful. Each of the full-thickness grafts was associated with considerable post-operative opacification which cleared slowly, progressing to complete clarity in only one case. As a result of these findings the authors concluded that, whereas donor material from the deep-freeze was suitable for lamellar grafts, its use could not be advised in perforating grafts. Subsequent observers have confirmed these results using similar and modified techniques of freezing (McNair and King, 1955; King, 1957).

Mr. Rycroft may well have solved this difficult and intriguing problem, and we look forward with great interest to the publication of his results. It is pertinent to observe, however, that in the case described Mr. Rycroft appears to have relied upon the protective action of 15 per cent. glycerol saline, a method which does not differ in principle from that used by Eastcott and others (1954); therefore, until such time as we are presented with new evidence, it should be stressed that the use of such material for routine perforating keratoplasty is entirely unjustified.

Yours faithfully,

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To the Editorial Committee of the BRITISH JOURNAL OF OPHTHALMOLOGY

SIRS,—Whilst the routine use of deep-freeze corneal graft material for all penetration grafts is not yet feasible, only the constant investigation and trial in selected cases under suitable conditions, with the accurate assessment of results, has justified future optimism for this method of preservation. Eastcott, Cross, Leigh, and North (1954) reported seven cases of human penetration grafts in which the grafts had been preserved by the deep-freeze method; two of these were stated to be completely successful, three were partially successful with no deleterious influence on vision, and there were two failures which were subsequently regrafted with success. These authors stated that the method had been found by them to give satisfactory results in the preservation of human corneal tissue for grafting.

In ophthalmology the future promise of the glycerol-saline method of preservation at -79°C ., first described by Polge, Smith, and Parkes (1949), rests on two established facts:

- (1) Lamellar grafts have proved to be entirely suitable when preserved in this fashion (Eastcott and others, 1954; King, 1957).
- (2) At least four successful cases to date of penetration grafts preserved in a similar manner have been carefully reported and followed up for many months after operation (Eastcott and others, 1954; Iliff, Wood, and Hollander, 1956; Leigh, 1954; Rycroft, 1957).

Nor does it follow that the opacification of the failures is necessarily due to the specific method of preservation, since many factors are involved and opacification can occur from these various causes in every method of preservation. For example, in the series previously quoted, one deep-freeze graft which had failed had been carried out in an eye in which a previous fresh graft had also failed "in the absence of any recognized post-operative complication". But the failure of the deep-freeze graft in this case was due to a condition which can occur in any type of penetration graft, namely the development of a posterior graft membrane; the graft tissue itself remained clear. Nevertheless, it is known that the behaviour of a deep-freeze full-thickness graft is distinctly different from that of a fresh graft and is associated with more prolonged oedema; this is a problem which remains to be solved.

The clinical approach to the solution must be further study of many selected cases, with variables controlled as far as possible in relation to the initial lesion, the type, age, and density of the scar, the degree of vascularization, the relationship of host and donor thickness, the age, sex, and general condition of the patient, the standardization of surgical and nursing technique, and the strict control of the sterility and method of donor collection. It is equally important that caution should also be exercised in the interpretation of the cases of failure in assessing the exact role which the method of preservation plays.

The essence of the laboratory problem is the behaviour of the endothelium and Descemet's membrane under the thermodynamic and biological stresses of low temperatures with subsequent reconstitution. Mr. Leigh suggests that the problem may have been solved, but I am sure he will agree that a solution is unlikely to be found in the operating theatre, but more likely in the laboratory; the ophthalmologist is only the builder and the biologist is the real architect.

Studying this particular problem, Ridge (1956) has shown that at pH 8.8 the rabbit endothelium is completely resistant to the toxic action of 10 per cent. glycerol, and that under these conditions the endothelium is completely protected against the harmful effects of freezing and thawing. At Bethesda, Draheim, McPherson, Evans, Perry, and Earle (1955) stated that preliminary tissue culture viability tests indicated that the rabbit cornea retains viability when preserved by this method.

At East Grinstead we are fortunate to work under the guidance of such authorities as Drs A. S. Parkes, Audrey U. Smith, and R. H. Billingham, and their valuable advice controls our deep-freeze investigations. They have pointed out the importance of such factors as the variability of temperature in a deep-freeze bank, the risk of carbon dioxide diffusion into any glass container which is not effectively sealed, the need for a slow rate of freezing and rapid thawing, and also that even yet the optimum percentage of glycerol is in doubt as well as the adequate time of soakage.

Surely, therefore, it is reasonable to expect that this problem will eventually be solved by the united efforts of the biologist, ophthalmologist, and pathologist, and that it will be possible to establish in every country of the world, where keratoplasty can alleviate blindness, Regional Banks in which adequate supplies of sterile and suitable corneal

donor material are readily available at the precise time when they are required.

This is the objective of the investigations of the deep-freeze method of preservation.

Yours faithfully,

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BOOK REVIEWS

Reactive Cellular Changes in the Cornea and Retina. (Reaktive Zellveränderungen in Hornhaut und Netzhaut.) By H. Pau. 1957. Samml. zwang. Abhild. Geb. Augenheilk., No. 13. Pp. 111, 100 figs, 82 refs.

This publication combines two separate studies under one general title. The first section deals with the reactions to injury and the regeneration of the cells and tissues of the cornea, and the second describes histological findings and other observations in retinal tears and detachments and places less emphasis on cellular reactions.

Most of the information on corneal cellular reactions has been derived from the rabbit cornea after experimental lesions and, whilst it is clearly the main intention of the author to describe the histological findings, it is regrettable that only the briefest mention is made of experimental procedures. However, the corneal changes after injury are described carefully in an interesting manner and with the aid of microphotographs which almost always illustrate well the points mentioned in the text. In many instances, a silver-staining technique was particularly useful for displaying details of nuclear structure. Frequent reference is made to corneal parenchymal cells which (as the author explains in a footnote on page 21) are not analogous to the parenchymal cells of other organs but are specialized connective tissue cells of the substantia propria. Small chromatin granules, usually five in number, were demonstrated in the nuclei of the so-called "fixed" cells of the cornea, and the important role of these cells in the healing of corneal wounds is described. It is claimed that the leucocytic cells in some forms of keratitis originate from corneal cells, the chromatic granules enlarging and becoming connected by bridges of nuclear material to form the segmented nuclei typical of some leucocytes. Similar changes were found in regenerating epithelial cells which proliferate and migrate to cover superficial lesions in the early stages of the healing process. The parenchymal cells of the cornea are also involved in the vascularization of corneal lesions and in the regeneration of corneal nerves, undergoing transformation to vascular endothelial cells and replacing Schwann cells.

The section dealing with retinal detachments contains descriptions of the histological